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Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium

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NOAA Technical Report NMFS SSRF-681

Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium

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CONTENTS

		Page
I.	Introduction and experimental design, by Anthony Calabrese, Ries S. Collier, and James E. Miller	1
II.	Uptake of cadmium by organs and tissues, by Richard A. Greig, Albert E. Adams, and Betty A. Nelson	5
III.	Changes in osmoregulation and oxygen consumption, by Frederick P. Thurberg and Margaret A. Dawson	11
IV.	Effects on the immune system, by Richard A. Robohm and Maureen F. Nitkowski	15
V.	Observations on the biochemistry, by Edith Gould and John J. Karolus	21
VI.	Histopathology, by Martin W. Newman and Sharon A. MacLean	27

SUMMARY ABSTRACT

The cunner, *Tautogolabrus adspersus,* was exposed to six concentrations of cadmium, as cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2} H_2O$), for 96 hr. At the end of this exposure period, tests of blood serum osmolality and gill tissue oxygen consumption were performed. High levels (48 ppm) of this metal resulted in abnormally high serum osmolality, and an exposure as low as 3 ppm reduced the normal rate of oxygen consumption. Both of these parameters may be related to observed tissue damage.

The histopathological effects of acute exposure of the cunner to cadmium were manifested in the kidney, intestine, hemopoietic tissue, epidermis, and gill. Few significant changes were noted in fish exposed to concentrations less than 48 ppm. The results implicate renal failure as the probable cause of death subsequent to acute exposure to cadmium.

Clearance of intracardially injected bacteria from the blood of cunners exposed to 12 ppm cadmium was examined. The rate of bacterial uptake in the cells of the liver and spleen was increased, but the bacterial death rate within these cells was decreased. Exposure of fish at 3 to 24 ppm failed to influence antibody production against sheep red blood cells.

The activity of two liver enzymes changed significantly with exposure to cadmium. Aspartate aminotransferase was lower in the exposed fish, and a magnesium-linked oxidoreductase in exposed fish required 10 times as much added magnesium to reach the same level of activity as in the control fish.

Chemical analyses were made for uptake and clearance of cadmium from exposed cunners. In the uptake study, cadmium residues averaged 8.5 times higher in liver than in gills. In the clearance study, substantial reductions in cadmium residues were found in the gills and blood of fish held in clean seawater for 6 wk after exposure to cadmium, as compared to fish sacrificed immediately after exposure. Muscle and carcass samples from the "cleared" fish showed little reductions in cadmium levels.

ACKNOWLEDGMENT

The authors of each section of this study thank Rita S. Riccio for her critical reading and typing of this manuscript.

Physiological Response of the Cunner, *Tautogolabrus adspersus*, To Cadmium. I. Introduction and Experimental Design

ANTHONY CALABRESE, RIES S. COLLIER, and JAMES E. MILLER[1]

INTRODUCTION

Like most metals, cadmium is stable and does not degrade in the environment. Thus, as increasing amounts of cadmium are refined, more and more of it is circulated in the environment, and increasing amounts may reach man. Cadmium becomes an air and water pollutant, through a variety of industrial processes, and is being used in increasing amounts by the storage battery, plastics, plating, and petroleum industries (U.S. Council on Environmental Quality, 1971). There is no evidence that cadmium is biologically essential or beneficial but, on the other hand, has caused severe human health problems (McKee and Wolf, 1971). Since cadmium salts are most likely to be found in estuarine areas that are important nursery areas for marine fish and shellfish larvae and juveniles, adult marine fish and shellfish are a potential source of cadmium in the human diet.

Relatively little is known about the effect of cadmium on aquatic animals, particularly those in the marine environment. Most studies on the effect of cadmium to aquatic species have been performed with freshwater forms. More recently, however, research emphasis has been directed toward the effect of cadmium salts on various marine organisms (Shuster and Pringle, 1968, 1969; Gardner and Yevich, 1969, 1970; Jackim, Hamlin, and Sonis, 1970; Eisler, 1971; Eisler, Zaroogian, and Hennekey, 1972; Calabrese et al., 1973). These studies have progressed from the more classical bioassay tests for simply determining TL_m (that concentration of toxicant causing 50% mortality) to those of physiological stress caused by sublethal levels of the pollutant being tested. Studies conducted at sublethal concentrations of a contaminant material so as to determine physiological damage to the organism concerned may be more important than mortality itself. The gradual elimination of valued marine species by low concentrations of toxicants is no less serious than instantaneous death of those species. In a sense, it is more serious because it is less likely to be obvious and to be traced to its source in time to permit recovery of the environment. Studies of physiological stress caused by sublethal levels of a toxicant generally require chronic or long-term exposure, but some physiological parameters can be examined in short-term tests. Parameters of physiological damage that can be examined by long-term exposure include growth, life span, reproductive success, adaptation to environmental stress, feeding and mating behavior, changes in respiration and osmoregulation, pathological effects, biochemical anomalies, and genetic alterations.

It is apparent that increases in human population and technological development are producing serious stresses on the marine environment, with a resulting decrease in its effective use. These events, plus natural events, are fostering conditions that diminish the harvest of marine resources. The National Marine Fisheries Service (NMFS), as part of the National Oceanic and Atmospheric Administration (NOAA), is concerned about the threat to marine life and is providing a national focus for marine research to generate the basic knowledge and understanding of marine environmental processes required for effective management of the marine environment and its resources.

The New York Bight, which is receiving international attention because of the large amount of waste material being dumped into it, borders the most heavily populated and industrialized complex in the country. Because the Middle Atlantic Coastal Fisheries Center of the NMFS is located within this geographical area, it is important that this Center undertake studies to determine the impact of man upon the living marine resources of this area. This Center, comprising laboratories in Sandy Hook, N.J.; Milford, Conn.; and Oxford, Md., has the facilities and scientists to undertake studies of this type. The present study was designed to determine the short-term (96 hr) physiological response of a local fish, *Tautogolabrus adspersus*, commonly known as the cunner, to cadmium. A multidisciplinary approach was used to determine the following: 1) uptake of cadmium into various tissues and organ systems; 2) changes in osmoregulation and oxygen consumption rates; 3) changes in enzymological patterns; 4) immune response to various antigens; and 5) induction

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of histopathological abnormalities. Results of these studies are reported in the following sections of this technical report.

METHODS AND MATERIALS

Collection and Conditioning

Cunners were collected in modified eel pots, a cylinder of ½-inch-mesh hardware cloth with a funnel in one end and a hinged door at the other end. Pots were baited with cracked hard clams, *Mercenaria mercenaria,* and fished in 10-25 feet of water in the Stratford to New Haven, Conn., area of Long Island Sound. Fish were transported to the laboratory in 15-liter polyethylene buckets and placed in tanks of flowing seawater at ambient temperature. Before being exposed to cadmium, the fish were transferred to tanks of recirculating artificial seawater (Zaroogian, Pesch, and Morrison, 1969), adjusted to 25 ppt salinity, and maintained at room temperature for at least 1 wk for acclimation. The fish were fed Purina Trout Chow[2] during this time, but were unfed for 2 days prior to and during the experiment.

Exposure

For exposure to cadmium, the fish were placed in glass aquaria filled to 60 liters, with artificial seawater (Instant Ocean), which was aerated throughout the entire exposure period. Cadmium, as cadmium chloride ($CdCl_2 \cdot 2½\ H_2O$), was added to test aquaria at concentrations of 0, 3, 6, 12, 24, and 48 ppm of Cd^{2+}. Stock solution for all tests was made up with reagent grade cadmium chloride dissolved in water at 50 g Cd^{2+} per liter and acidified to a pH of 2.5 to maintain stability. Proper aliquots of the cadmium stock solution were added immediately prior to the addition of the artificial seawater to obtain desired cadmium concentrations, and aeration was begun a few minutes prior to the introduction of test fish. Temperatures ranged from 21° to 25°C and pH levels remained between 7.3 and 7.6 during the entire study. The above five concentrations of cadmium were tested in duplicate, with two aquaria serving as controls, in each of a series of seven tests. Four cunners were placed in each aquarium and were observed daily throughout the 96-hr exposure period, and dead fish were removed each day. At the termination of each test the fish were made available to resident scientists at the Milford laboratory; in addition, specimen samples were prepared for histopathological examination by scientists at the Oxford laboratory, Oxford, Md. Supplementary tests of the same design were subsequently performed for those research projects needing further samples.

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Of a total of 500 fish tested, 126 were sampled fo weight and length, averaging 45.2 g in weight an 146.3 mm in length, with a range in length from 115 t 170 mm.

RESULTS AND DISCUSSION

Although the intent of this study was to determin the physiological response of cunners exposed to sub lethal levels of cadmium, rather than to determin TL_m, some mortality data were nevertheless obtained Concentrations of cadmium (in ppm water) teste and percent mortality (in parentheses) follow: con trols 0 (1.8%), 3 (3.5%), 6 (5.4%), 12 (1.8%), 2 (10.7%), and 48 (26.8%). It is obvious from these dat that cunners can tolerate high concentrations of cad mium for at least 96 hr. It was also noted in othe phases of this study, however, that those fish expose to 48 ppm of cadmium for 96 hr and then placed i clean seawater all died within a few days. Eisle (1971) reported that the tautog, *Tautoga onitis,* a fis in the same family as the cunner, can also tolerat high levels of cadmium for a short duration. He als reported that the sheepshead minnow, *Cyprinodo variegatus,* and the mummichog, *Fundulu heteroclitus,* had TL_{50} values of 50 and 55 ppm respectively, for a 96-hr exposure to cadmium Although it appears that marine teleosts are relativel resistant to cadmium, it will be apparent from th sections that follow that cadmium does, in fact, caus physiological stress at sublethal concentrations.

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Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium. II. Uptake of Cadmium by Organs and Tissues

RICHARD A. GREIG, ALBERT E. ADAMS, and BETTY A. NELSON [1]

ABSTRACT

Cadmium uptake and clearance data were obtained on cunners, *Tautogolabrus adspersus*, exposed to various concentrations of this metal in artificial seawater.

In the uptake study, cunners were exposed to 0, 3, 6, 12, 24, and 48 ppm cadmium in seawater for 4 days. Cadmium residues averaged 8.2 times higher in livers than in gills. At the 48 ppm cadmium exposure level, the livers averaged 195 ppm, as compared to 33.5 ppm for gills (wet weight values).

In the clearance study, cunners were exposed to 24 ppm cadmium in seawater for 4 days, after which time half of the fish were placed in clean flowing seawater for 1 mo and half were sacrificed immediately to determine initial cadmium residue concentrations. Gill, liver, blood, muscle, and carcass samples were analyzed. Substantial reductions in cadmium residues were found in the gills and blood of fish held in clean seawater, as compared to samples from fish sacrificed immediately after exposure to cadmium. Liver samples produced variable results: livers of fish held in clean seawater for 1 mo contained 62-155 ppm cadmium for four fish and 5-11 ppm for three fish, as compared to 30-117 ppm for livers from eight fish sacrificed immediately after exposure to cadmium. Muscle and carcass samples from the "cleared" fish showed very little reduction in cadmium levels.

INTRODUCTION

Freshwater and marine organisms have the ability o concentrate metals far in excess of the levels found n the waters they inhabit. Mollusks and other nellfish, in particular, selectively concentrate hemical materials (including metals) up to many undreds of times the levels in their environment Pringle et al., 1968).

There are three major sources for uptake of metals y water-inhabiting organisms:

1. The water column (the metals are dissolved in the water);
2. Particulate matter (the metals are adsorbed to particles suspended in the water column); and
3. Food (the metals are incorporated in the material the organism consumes).

There are relatively few reports in the literature ealing with the uptake of cadmium by marine rganisms. Of those few marine animals studied for admium uptake, mollusks have received the most ttention and finfish the least (Pringle et al., 1968; huster and Pringle, 1969; Eisler, Zaroogian, and ennekey, 1972). Several investigators have studied the toxic effects of cadmium in both freshwater and marine finfish, but these workers did not include information on the uptake of cadmium (Ball, 1967; Gardner and Yevich, 1969, 1970; Roberts, 1963; Eisler, 1971).

Our objective was to obtain data on the uptake of cadmium by liver and gill tissues of cunners exposed to solutions of $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$ in artificial seawater. In addition, information was obtained on the extent of clearance of cadmium from various tissues and organs of cunners that were returned to clean seawater after exposure to cadmium.

METHODS AND MATERIALS

Fish Holding

Uptake study.—The methods of exposure of the cunner to various concentrations of cadmium chloride in artificial seawater for the uptake study are described in Part I of this collaborative report.

Clearance study.—An independent study was conducted to determine the extent of clearance of cadmium from various tissues of the cunner, after the exposed fish were held in clean seawater. Sixteen fish were exposed to 24 ppm cadmium (as $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$) in artificial seawater for 96 hr, and eight fish were maintained as controls. The fish weighed from 28-89 g

[1] Milford Laboratory, Middle Atlantic Coastal Fisheries Center, ational Marine Fisheries Service, NOAA, Milford, CT 06460.

($\bar{x}$ = 62.3 g). After the 96-hr exposure, eight of the exposed fish and four control fish were sacrificed to determine cadmium residues in various parts of the fish. The remaining eight cadmium-exposed fish and four control fish were placed in flowing seawater for 1 mo, after which time they were sacrificed for cadmium analysis.

Sampling Procedures

Uptake study.—For the uptake study, data were collected from three separate experiments, in each of which five different concentrations of cadmium were used, plus a control. A single pooled sample was made of the livers from four to five fish per exposure level. The same sampling procedure was followed with the gills, which were rinsed in clean seawater immediately after dissection to avoid possible adherence of cadmium chloride to the gill surfaces.

Clearance study.—Muscle, gills, liver, red blood cells, serum, and carcass were analyzed for cadmium residues. The sampling was as follows:

Muscle.—Paired fillets were taken from each fish, skinned, and ground and combined into a single sample per fish. The skin was added to the carcass sample (described below).

Gill and liver.—Samples were taken as described above for the uptake study, except that the samples were analyzed individually.

Red blood cells and serum.—Blood samples were pooled from four fish per treatment, except in the case of the cadmium-exposed fish held 1 mo in clean running seawater; only seven fish survived, and their blood pools represented four and three fish, respectively.

Whole blood was taken from the cunner by heart puncture, placed in a test tube, and allowed to clot at room temperature for 45-60 min. The serum was removed from the clot and centrifuged at 350-500 XG for 10 min. The clarified serum was frozen-stored until analysis. For the red blood cells samples, the cellular residue remaining after centrifugation was combined with the clot.

Carcass.—The remainder of the fish after removal of the samples described above was called the carcass, and included the skin removed from the fillets.

Chemical Analyses

For analysis of cadmium in cunner tissues, samples were placed in 50-ml glass beakers, dried at 110°C for 18 hr, and heated over a Bunsen burner to char the tissue. The samples were brought to 400°C in a muffle furnace, removed after 1 hr at that temperature, and cooled; a small amount of concentrated HNO_3 was added to wet the ash, and the samples were returned to the muffle furnace at room temperature and brought to 400°C again. This process was repeated until only a white residue remained in the beakers, usually after 3-5 additions of HNO_3. The residue was rinsed from each beaker with 10% HNO_3 and filtered through Whatman [2] No. 2 paper. The filtrate was brought to a final 10-ml volume and subsequently analyzed with an atomic absorption spectrometer employing a deuterium background corrector (Perkin Elmer[2] Model 403).

RESULTS

Uptake Study

Cadmium accumulation was far greater in the liver than in the gills (Table 1). Cadmium concentrations averaged 8.2 (range 3-15) times higher in liver than in gill tissue for all concentrations tested. Although there was substantial variation in results for the three uptake experiments, the averaged data show a nearly linear relation for cadmium concentrations in liver versus cadmium exposure levels (Fig. 1). Variation in liver-cadmium concentration was greatest at 24 and 48 ppm exposure levels, and least at 3, 6, and 12 ppm levels.

Uptake of cadmium into gill tissues was curvilinear in form, as shown in a plot of cadmium concentrations in gills versus cadmium exposure levels (Fig. 2). Cadmium concentrations in gill tissue for 3 and 6 ppm

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

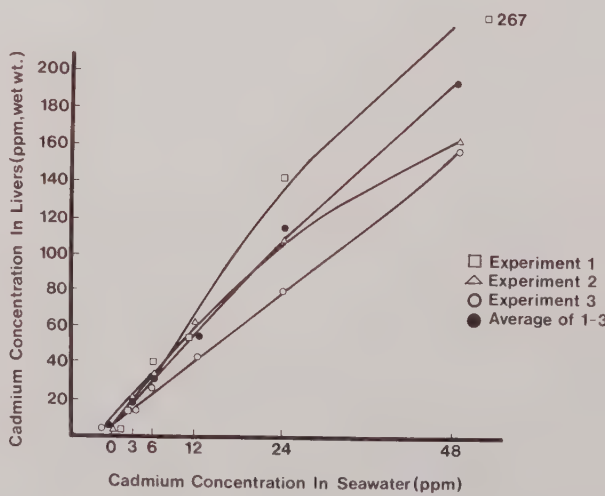
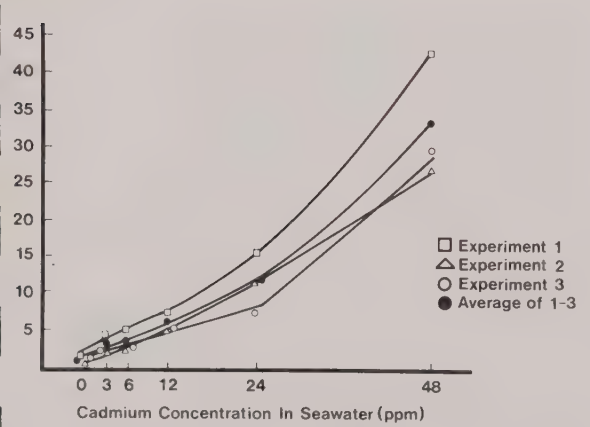


Figure 1.—Uptake of cadmium by the livers of cunner held 96 hr in various concentrations of cadmium (as cadmium chloride) in artificial seawater.

Table 1.—Uptake of cadmium by livers and gills of cunners, *Tautogolabrus adspersus*, exposed for 96 hr at various concentrations of cadmium, as $CdCl_2 \cdot H_2O$, in artificial seawater.

Concentration of cadmium in artificial seawater (ppm)	Concentrations of cadmium in tissues							
	Individual experiments				Individual experiments			
	Av.	1	2	3	Av.	1	2	3
	ppm wet weight basis[1]				*ppm dry weight basis*[1]			
	LIVER TISSUE							
0	1.2	0.95	1.65	0.85	5.5	3.6	6.7	3.2
3	16.0	13.5	21.5	13.0	54.5	41.0	75.0	47.5
6	34.5	39.0	36.5	27.5	119.5	125.0	131.0	102.0
12	55.0	54.5	65.0	45.0	198.7	182.5	236.0	177.5
24	110.7	143.0	109.0	80.0	390.0	454.0	386.5	329.5
48	195.0	267.0	160.5	157.0	761.3	928.0	744.0	611.5
	GILL TISSUE							
0	1.1	1.1	0.9	1.3	5.4	5.4	5.0	7.3
3	3.0	4.3	2.3	2.5	16.5	21.5	11.5	16.5
6	3.4	5.1	2.4	2.7	17.5	28.0	12.5	13.0
12	6.3	7.5	5.8	5.6	31.8	38.5	28.0	29.0
24	11.9	16.0	12.0	7.8	66.5	88.5	60.5	44.0
48	33.5	43.0	27.5	30.0	171.3	226.5	135.0	152.5

[1] Liver and gill tissues from four to five fish per exposure level were composited and analyzed in duplicate for each experiment. The values shown for individual experiments are averages of the duplicate analyses.



igure 2.—Uptake of cadmium by the gills of cunner held 96 hr various concentrations of cadmium (as cadmium chloride) in rtificial seawater.

admium exposure levels were virtually the same, and noderate increases occurred at 12 and 24 ppm levels. here was a very sharp increase, however, at the 48 pm level, which may be related to physiological gill amage. Thurberg and Dawson (this report, Part III) ound a marked depression in oxygen consumption tes of gill tissues, as well as a breakdown of osoregulation in cunners exposed to 48 ppm cadmium.

Clearance Study

Substantial reductions in cadmium residues were found in the gills, red blood cells, and serum of cadmium-exposed fish held in clean running seawater for 1 mo after exposure. In contrast, muscle and carcass samples of these fish showed very little reduction in cadmium residues, as compared to fish examined immediately after exposure to cadmium (Table 2).

Liver samples produced variable results (Table 2). Cadmium concentrations in livers of fish examined immediately after exposure varied from 30 to 117 ppm ($\bar{x}$ = 64.2), a nearly 4-fold difference. Cadmium concentrations in livers of fish held 1 mo in clean running seawater after exposure, varied only 5-11 ppm ($\bar{x}$ = 10) in three of the fish, but varied from 62 to 155 ppm ($\bar{x}$ = 92) in the other four fish.

In spite of the pooling of blood samples (which reduced the number of possible observations), cadmium concentrations in both serum and red blood cells were as variable as those observed in the individual liver samples (Table 2). All blood pools from "cleared" fish, however, had substantially lower levels of cadmium than those from fish sacrificed immediately after exposure to cadmium.

Gill tissues showed a greater clearance of cadmium than did the other tissues examined. Gills of fish sacrificed immediately after exposure contained 6.2-10.6 ppm cadmium ($\bar{x}$ = 8.1), and gills of fish held in

Table 2.—Clearance of cadmium from organs and tissues of cunners, *Tautogolabrus adspersus,* held in flowing natural seawater for 1 mo after a 96-hr exposure to 24 ppm cadmium, as $CdCl_2 \cdot 2\frac{1}{2} H_2O$.

Organ or tissue	Cadmium Concentration	
	Immediately after exposure Average (range)	After 1 mo in clean seawater Average (range)
	ppm (wet weight)	
	TEST FISH	
Flesh	0.17 (0.11 - 0.22)	0.12 (0.08 - 0.22)
Liver	64.2 (30.5 - 117.2)	92.0 (62.0 - 155.0)[1]
		10.0 (5.0 - 11.0)[1]
Gills	8.1 (6.2 - 10.6)	3.5 (2.8 - 4.7)
Red blood cells	6.6 (5.2 and 8.0)	1.8 (0.8 and 2.8)
Serum	5.9 (5.9 and 6.0)	1.5 (0.7 - 2.3)
Carcass	4.8 (0.9 and 6.2)	3.5 (2.8 - 4.2)
	CONTROL FISH	
Flesh	0.06 -	0.05 -
Liver	0.7 (0.6 and 0.8)	1.0 -
Gills	0.4 -	0.3 -
Red blood cells	0.4 -	0.4 -
Serum	0.4 -	0.5 -
Carcass	0.09 (0.08 and 0.10)	0.12 -

[1] The livers of 4 fish had cadmium concentrations in the range of 62-155 ppm, whereas the livers of 3 other fish had cadmium concentrations in the range of 5-11 ppm.

clean running seawater for 1 mo after exposure contained 2.8-4.6 ppm cadmium.

Concentrations of cadmium found in tissues of control fish (Table 2) were all less than 1 ppm.

DISCUSSION

In the literature, studies of the uptake of cadmium and other metals by marine animals deal predominantly with shellfish. Pringle et al. (1968), who studied the uptake of five metals by several species of shellfish, examined only the soft-shell clam, *Mya arenaria,* for cadmium uptake. Clams exposed to 0.05 ppm cadmium (nitrate) in flowing seawater for 70 days accumulated 8 ppm (whole-body wet weight), and clams exposed to 0.1 ppm for 56 days accumulated 9 ppm. Shuster and Pringle (1969) exposed Eastern oysters, *Crassostrea virginica,* to cadmium (nitrate) in flowing seawater for 20 wk. At a 0.1 ppm exposure level, oysters accumulated 90-100 ppm cadmium (whole-body wet weight) within 13 wk, whereas at a 0.2 ppm exposure level, the animals accumulated the same concentrations of cadmium within 8-10 wk. After only 1 wk at 0.1 and 0.2 ppm exposure levels, the oysters accumulated 7-24 ppm cadmium, with no apparent difference due to exposure levels.

Eisler et al. (1972) studied cadmium uptake by Eastern oysters; American lobsters, *Homarus americanus;* bay scallops, *Aquipecten irradians;* and mummichog, *Fundulus heteroclitus.* The animal were held for 21 days in flowing seawater containin 10 ppb cadmium ($CdCl_2 \cdot 2\frac{1}{2} H_2O$). Accumulation o cadmium was highest in the oysters, with 1.49 ppm (whole-body wet weight) in the exposed animals an 0.33 ppm in control animals. The mummichog ha whole-body residues of 0.48 ppm cadmium for ex posed fish and 0.33 ppm in control fish.

For comparison with these studies, a combined es timate of all data was made for whole-body residues i cunners exposed to 24 ppm cadmium for 96 hr (se clearance study, Table 2). The whole-body residue were calculated to be 2-4 ppm (wet weight) for ex posed fish and 0.1-0.2 ppm for control fish. Although direct comparison of these data with those cited abov for oysters is not possible, it would appear that cunne accumulates cadmium to a much lesser extent tha the oyster.

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Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium. III. Changes in Osmoregulation and Oxygen Consumption

FREDERICK P. THURBERG and MARGARET A. DAWSON[1]

ABSTRACT

The cunner, *Tautogolabrus adspersus*, was exposed to various concentrations of cadmium, as cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2}\ H_2O$), for 96 hr. At the end of this exposure period tests of blood serum osmolality and gill tissue oxygen consumption were performed. High levels (48 ppm) of this metal resulted in an abnormally high serum osmolality and an exposure as low as 3 ppm reduced the normal rate of oxygen consumption. Both of these parameters may be related to observed tissue damage.

INTRODUCTION

Cadmium, which is neither essential nor beneficial to aquatic organisms (McKee and Wolf, 1971), has been detected in increasing amounts in the tissues of a number of such animals (Mullin and Riley, 1956; Pedeen et al., 1973). The use of cadmium in a variety of industrial processes has increased in recent years, making this metal an immediate concern as an environmental pollutant (U.S. Council on Environmental Quality, 1971; Dean, Bosqui, and Lanouette, 1972). A number of investigators have demonstrated the toxicity of this metal to aquatic animals (Eisler, 1971; Calabrese et al., 1973; Collier et al., in press); however, little is known of the sublethal effects of cadmium on finfish. Other metals have been shown to alter serum osmolality and respiration of freshwater fish (McKim, Christensen, and Hunt, 1970; Lewis and Lewis, 1971). Few experiments in this area have been conducted with metals and marine fish and fewer still with cadmium as the test pollutant. The present study was undertaken to determine the effect of cadmium on osmotic regulation and oxygen consumption in the cunner, *Tautogolabrus adspersus*.

METHODS AND MATERIALS

Cunners were exposed to 0, 3, 6, 12, 24, and 48 ppm cadmium for 96 hr by the method of Calabrese, Collier, and Miller (this report, Part I). At the end of this exposure period, a blood sample was drawn by heart puncture using a scalpel and a disposable Pasteur pipette. Pooled blood samples from three to four fish per cadmium concentration were collected in chilled 15-ml centrifuge tubes and spun at 1,720 × *g* for 20 min at 4°C. The osmolality in milliosmoles per Kg H_2O (mOsm) of 0.2-ml serum samples was read on an Advanced 3L Osmometer.[2] Gill tissues from these same fish were dissected out and placed in 15-ml Warburg-type flasks chilled on ice; each flask contained 5 ml of cadmium-treated seawater from the tank from which the fish were removed. Oxygen consumption was monitored over a 4-hr period in a Gilson Differential Respirometer at 20°C. Oxygen consumption rates were calculated as microliters of oxygen consumed per hour per milligram dry weight of gill tissue (μl/hr/mg) corrected to microliters of dry gas at standard temperature and pressure.

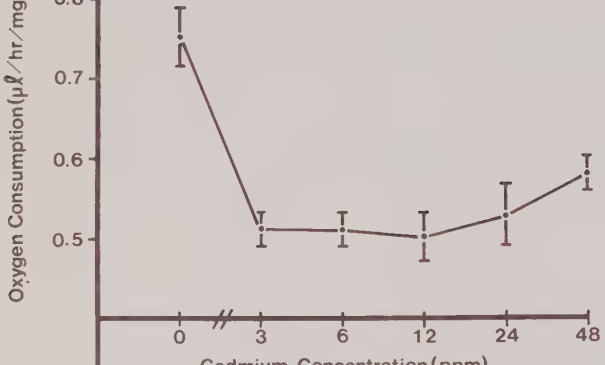
RESULTS

Cunners exposed to 3-24 ppm cadmium for 96 hr showed no change in serum osmolality from the normal value of approximately 340 mOsm determined in control fish. This value is lower than that of the surrounding seawater (630 mOsm). Osmoregulatory difficulties were noted in fish exposed to 48 ppm. Serum osmolality in these fish rose to an average value of 390 mOsm. These data are presented in Figure 1; each point on the curve represents the mean of six pooled samples, one from each of six exposures at a given cadmium concentration.

Cadmium reduced the gill tissue oxygen consumption rates at all concentrations tested. A normal rate of 0.750 μl/hr/mg was reduced to approximately 0.510 μl/hr/mg after exposure to cadmium at concentrations

[1] Milford Laboratory, Middle Atlantic Coastal Fisheries Center, National Marine Fisheries Service, NOAA, Milford, CT 06460.

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.



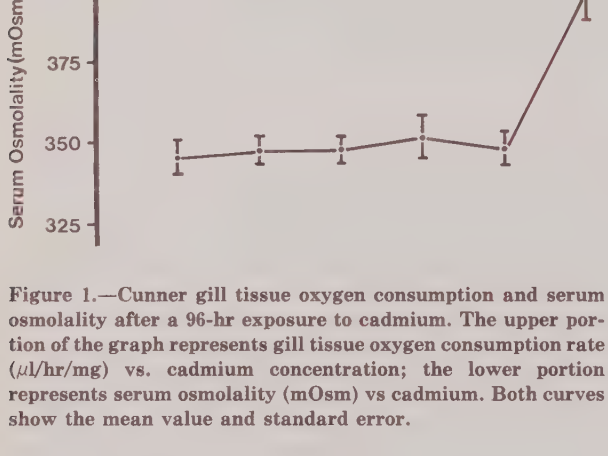


Figure 1.—Cunner gill tissue oxygen consumption and serum osmolality after a 96-hr exposure to cadmium. The upper portion of the graph represents gill tissue oxygen consumption rate (μl/hr/mg) vs. cadmium concentration; the lower portion represents serum osmolality (mOsm) vs cadmium. Both curves show the mean value and standard error.

of 3, 6, 12, and 24 ppm cadmium. Oxygen consumption was slightly higher (0.580 μl/hr/mg) after exposure to 48 ppm, the same concentration at which osmoregulatory stress was observed. These results are presented in Figure 1; each point on the curve represents the mean value of gill tissue oxygen consumption of 18 fish.

DISCUSSION

Marine teleosts maintain a normal blood serum osmolality considerably below that of the surrounding medium (Krogh, 1965; Parry, 1966). In the present study cunner serum osmolality rose considerably above its normal level after a 96-hr exposure to 48 ppm cadmium. Exposures below this level did not alter serum osmolality. Teleost kidneys excrete salts and thus maintain a normal osmotic concentration. Newman and MacLean (this report, Part VI) detected gross pathology in the kidneys of certain cunners used in this study. They reported that kidneys of cunners exposed to 48 ppm cadmium for 96 hr were nearly nonfunctional, while those exposed to cadmium concentrations below 48 ppm appeared normal. Gill and gut tissues are also involved in osmoregulatory function (Krogh, 1965; Prosser and Brown, 1961). Other investigators have reported gill and kidney tissue damage in marine teleosts after exposure to cadmium (Gardner and Yevich, 1970; Eisler, 1971), and Newman and MacLean (this report, Part VI) noted some gill and gut damage in cadmium-exposed cunners. Osmoregulatory difficulty at 48 ppm is, therefore, apparently due to kidney failure, although gill and gut damage may be contributory.

Gill tissue oxygen consumption was depressed after exposure to 3-48 ppm cadmium. The slight rise (although still well below the normal level) in oxygen consumption at 48 ppm was attributed to increased osmoregulatory stress at that concentration. The depression of oxygen consumption may have been due to gill damage. Newman and MacLean (this report, Part VI) noted gill tissue abnormalities in fish exposed to cadmium, and Ledgerwood and Brown (1973) reported cadmium-induced aneurysms in the gill lamellae of threespine sticklebacks, *Gasterosteus aculeatus*. Greig, Adams, and Nelson (this report, Part II) found elevated levels of cadmium present in gill tissues of all exposed cunners examined.

In summary, the results of this study demonstrated two physiological effects of cadmium on the cunner. High levels (48 ppm) of this metal resulted in an abnormally high serum osmolality, and an exposure as low as 3 ppm reduced the normal rate of oxygen consumption. Both of these parameters may be related to observed tissue damage.

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Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium. IV. Effects on the Immune System

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ABSTRACT

Two elements of the immune system in cunners, *Tautogolabrus adspersus*, were examined after 96-hr exposure to cadmium: 1) clearance of intracardially injected bacteria from the bloodstream and 2) ability to produce antibody against intraperitoneally injected sheep red blood cells (SRBC). Exposure to 12 ppm cadmium increased the rates of bacterial uptake in phagocytes of the liver and spleen but significantly decreased the rates of bacterial killing within these cells. Exposure of fish at 3 to 24 ppm cadmium failed to influence antibody production against SRBC. These results indicate that cadmium affects one aspect of cellular immunity but not humoral immunity in cunners. This effect may increase susceptibility to infection.

INTRODUCTION

There is evidence that cadmium poisoning in teleosts disrupts respiratory processes (Schweiger, 1957; Mount and Stephan, 1967) and damages kidneys (Gardner and Yevich, 1970) and gills (Mount and Stephan, 1967; Gardner and Yevich, 1970). The exact mechanisms of these effects are unknown, although some evidence in liver, kidney, and other tissues of mammals (Simon, Potts, and Gerard, 1947) and fish (Jackim, Hamlin, and Sonis, 1970) indicates that enzyme systems are inhibited. If there is inhibition in rapidly growing or metabolizing cells, then cells of the immune system in fish may also be affected. Identification of such effects, in addition to being useful as indicators of toxicity, may, in part, explain environmental mortalities of fish. For example, if a pollutant can inhibit production of antibody by the lymphocytes or in some way reduce the effectiveness of reticuloendothelial system phagocytes, fish may become susceptible to infection. A report by Pippy and Hare (1969) linking bacterial infection of salmon with sudden spikes of copper and zinc levels in a Canadian river is indicative that metal pollution may indeed lower fish immunity.

The following investigation was undertaken to determine whether short-term exposure of the cunner to sublethal cadmium levels would limit antibody production or reduce clearance of bacteria from the blood, spleen, and liver.

METHODS AND MATERIALS

Fish Holding and Cadmium Exposure

Cunners, *Tautogolabrus adspersus*, were captured from the wild, acclimated to artificial seawater, and exposed to 3 to 48 ppm Cd^{2+} (as $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$), as described by Calabrese, Collier, and Miller (this report, Part I). Some fish were immunized (as described below) with sheep red blood cells (SRBC), removed from Cd^{2+} treated water, and held an additional 10 days at ambient temperature (23 ± 2°C). In two experiments these immunized fish were held in 20-gal fiber glass aquaria containing recirculating seawater under constant charcoal filtration. The fish (with dorsal spine, pectoral fin, or pelvic fin clipped to denote levels of cadmium treatment) were held nine or less per tank, and the water changed every second day. In two later experiments with immunized fish, fins were no longer clipped, and fish were held in running seawater (20°-23°C) in 500-gal fiber glass tanks partitioned off with polyethylene mesh.

Some fish were exposed to 12 ppm Cd^{2+} treated water then removed and held for 1 or 2 days in the running seawater system. These fish were challenged by intracardial injection of bacteria, as described below.

Immunization and Collection of Antisera

Sheep red blood cells in Alsever's solution were washed in phosphate buffered physiological saline (PBS), pH 7.2, until the supernatant was free of hemoglobin and then diluted in PBS to 0.5% suspension (packed cell volume/volume PBS). Fish were weighed and injected intraperitoneally with 0.2 ml of the suspension per 40-g fish (i.e., 2.5×10^{-5} ml packed SRBC/g fish weight). SRBC injections were made at 0

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hr, at 0 minus 24 hr or at 0 plus 24 hr relative to the start of the 96-hr cadmium-exposure period. Second "booster" injections of SRBC were given 7 days after each initial injection. Serum was drawn from each fish 6 or 7 days after the second SRBC injection. Blood was removed from the ventral aorta using a Pasteur pipette equipped with a small, rubber finger bulb after nicking the artery with a scalpel blade. Blood was allowed to clot at room temperature and the clots to retract at 4°C. After removing red cells from the serum by centrifugation, sera were stored at −25°C until assayed.

Hemagglutination Assay

The microtiter system of the Cook Engineering Co., Alexandria, Va., was used in making dilutions and setting up hemagglutination assays. Serial twofold dilutions of fish serum were made in PBS, pH 7.2, containing normal rabbit serum (NRS) at 1/100 concentration (the rabbit serum was preabsorbed with SRBC). Washed SRBC were suspended to 1% concentration (v/v) in the PBS-NRS diluent and added to each serum dilution (0.025-ml serum plus 0.025-ml SRBC suspension). Controls included normal nonimmune fish serum, known positive serum from immunized fish and PBS alone. After 2-hr incubation at room temperature and overnight at 4°C, the titers of each serum were read by examining the degree of SRBC agglutination based on a 0 to 4+ rating scale. The last dilution causing a 2+ agglutination of SRBC was taken as the titer. A 2+ rather than a 1+ endpoint was used because it gave more reproducible results. Heat inactivation was not done because it created a gel in the serum. Hemolysis of red cells was not a problem because it occurred only after 48-hr incubation—a time long after final readings had been made.

Growth and Injection of Bacteria

Cells of *Bacillus* sp (biochemical tests consistent with *Bacillus cereus*) were grown well into stationary phase culture (72 hr) in Trypticase soy broth. The medium in the culture vessel was constantly agitated by an air-driven magnetic stirrer while the vessel was held in a water bath at 37°C. After incubation the culture consisted of about 70% single cells and 30% cells attached in pairs (with less than 0.1% spores) by phase-contrast microscopy. Cells were diluted in physiological saline, containing 0.1% peptone, and counted by the pour plate method in Trypticase soy agar. The following day the bacteria were washed 2× at 3°C with 0.15 *M* PBS, pH 7.2, and resuspended to about 5×10^6 cells/0.1 ml in PBS based on the counts of the previous day.

Fish which had been held for 24 or 48 hr in running seawater after termination of 96-hr exposure to 0 ppm or 12 ppm Cd^{2+} were injected intracardially on a vol/wt basis with the bacterial suspension. For exam ple, a 40-g fish received 0.1 ml, a 60-g fish receive 0.15 ml, etc. Actual numbers of viable bacteria in jected per 40-g fish varied from 5×10^5 to 5×1 because of some cell loss and death during washing i the PBS diluent. Actual numbers were determined b bacterial counts on the suspension after injecting a the fish on a particular day.

Measurement of Bacterial Clearance

Intracardially injected fish were placed in 4-ga polyethylene pails containing seawater at ambien temperature (23°C). After 30 or 90 min, bacteria counts were made of the blood, liver, and spleen usin the following procedures: Blood was removed from th ventral aorta with a premarked, heparinized Natelso blood collecting pipette; blood was drawn to the mar (0.125 ml) using mouth suction on the end of a shor rubber tube with mouthpiece (after first wetting th heparin with a small amount of blood). Blood wa diluted immediately into a tube of physiologica saline containing 0.1% peptone. In order to minimiz the amount of standing blood (containing bacteria) i the organs, aspiration of blood was continued unti the fish was bled dry. The liver and spleen wer removed, weighed to three places, and each diluted i an aqueous solution of 0.5% peptone. In many in stances, blood from the pericardial space spilled ont the liver during removal of that organ. When thi happened, the liver was washed with sterile, distille water and blotted on sterile paper toweling prior t weighing. Each liver and spleen was ground in sterile, motor driven tissue grinder with teflon pestl and further diluted serially in 0.1% peptone-salin diluent. Plate counts of the blood, liver, and splee were made in Trypticase soy agar within 10 min o organ removal. Values were recorded as percent of th initial bacterial dose present in each organ Calculations for total bacteria in the blood strean were based on a blood volume of 3% of the fish bod weight (this was approximated from values given b Thorson, 1961).

RESULTS

Antibody Response to SRBC Injections

In order to examine the possibility that cadmiun could affect protein formation or cell division in newl produced, immunocompetent cells, fish were give priming doses of SRBC followed by a second antige dose 7 days later. Production of antibody was mea sured by ability of fish serum to agglutinate washe SRBC. Table 1 compares the reciprocal hemagglutina tion titers of cadmium-treated fish and fish receivin no cadmium during the 96-hr holding period. Al though antigen was injected into fish on the day be fore, the day of, or the day after the start of cadmiun

Table 1.—Serum hemagglutination titers of fish immunized by intraperitoneal injection of sheep red blood cells (SRBC) at the time of 96-hr cadmium exposure. Sera were drawn 2 wk later (1 wk after a second SRBC injection).

	Cadmium concentration (ppm)					
	0		3-6		12-24	
	No. tested	Titer[1]	No. tested	Titer	No. tested	Titer
Immunized fish	12	33±11	23	29±6	17	30±5
Nonimmunized fish	9	6±1	12	3±1	12	6±1

[1] Titer is shown as the reciprocal of the mean serum dilution±standard error.

reatment, no differences were noted in eventual mount of antibody produced; therefore, the data n the table are grouped as though all antigen injections were given on the same day.

It may be seen from the table that treatment of fish vith low doses (3-6 ppm) or high doses (12-24 ppm) of Cd^{2+} did not cause any significant differences in antibody response over those produced by fish not exposed to Cd^{2+}. The table also shows that fish had a natural, low-level agglutinin to SRBC which was distinguishable from immune agglutination by its lower iter. About half of the fish tested had no natural SRBC agglutinins. All fish treated with 48 ppm cadmium died within the 2-wk holding period even hough held in fresh, Cd^{2+}-free water.

Effects of Cadmium on Bacterial Clearance

Experiments were run to determine whether admium exposure would affect another aspect of immunity in cunners, namely, clearance of bacteria by hagocytic cells of the reticuloendothelial system. The two primary elements of this system, the liver nd spleen, were examined. Bacteria injected into the loodstream via intracardial route were counted after 0 and 90 min for their remaining levels in the blood, or quantities picked up in the liver and spleen, and by subtraction) for quantities killed within the 30- nd 90-min time intervals. Five experiments were run n which a total of 10 or 11 fish were used for each of he following four variables: 0 ppm Cd^{2+} at 30 min, 2 ppm Cd^{2+} at 30 min, 0 ppm Cd^{2+} at 90 min, and 2 ppm Cd^{2+} at 90 min. Fish caught and used in he warm summer months had significantly greater learance rates than those caught and used in the utumn; however, the relationships of the 30- and 90- nin effects and the 0 ppm and 12 ppm Cd^{2+} effects vere approximately the same. Therefore, to get a epresentative mean (one in which the low count experiments would carry as much weight as high count xperiments), each value within an experiment was multiplied by a factor for that experiment according to the following example:

$$\text{Factor}_i = \frac{\text{mean of all values}}{\text{mean for experiment \#1}}.$$

This allowed each experiment to be representative in calculating the mean while preserving all the treatment effect relationships within that experiment.

Figure 1 depicts the effects of Cd^{2+} on clearance of bacteria. Wilcoxon's signed rank test for paired observations was used to determine significance of effects

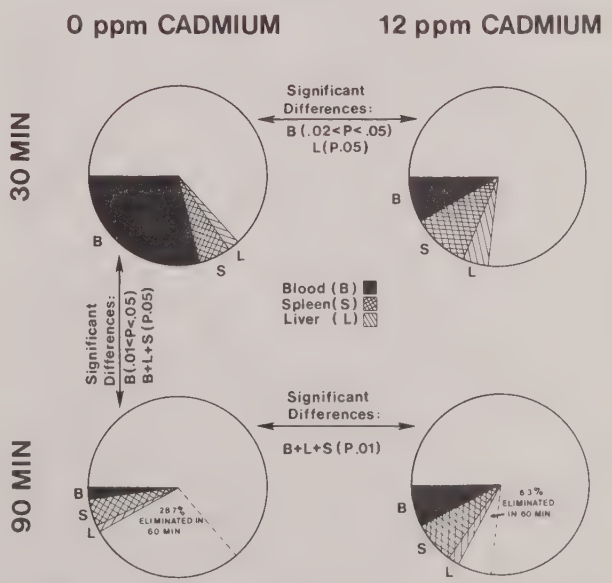

Figure 1.—Bacterial clearance from the blood, liver, and spleen. Diagrams represent mean percent of bacteria remaining (based on number of bacteria injected) after the 30- or 90-min time intervals. Values from 10 or 11 fish were used for each variable.

between Cd^{2+} exposure and no Cd^{2+} exposure (Cd^{2+} treated fish were matched by weight ±30% with non-treated fish within each experiment). Wilcoxon's two-sample test for unpaired observations was used to test significance between 30- and 90-min time intervals. Figure 1 shows that within the first 30 min, Cd^{2+} exposed fish clear bacteria from the blood stream more rapidly and take up greater numbers of bacteria in the liver and spleen than fish not exposed to Cd^{2+}. Differences in counts in the blood and liver between Cd^{2+} treated and nontreated fish are significant. However, counts in the cadmium-treated fish change very little during the next 60 min, i.e., only 6.3% additional cells are eliminated. By contrast, the counts in fish not exposed to cadmium continue to decrease in the blood, liver, and spleen between 30 and 90 min. Values for the blood and the total remaining cells in the blood + liver + spleen are significantly different; 28.7% of the initial bacterial load is eliminated (or killed) within the 60-min time interval. Thus, at 90 min the total remaining bacteria in the non-Cd^{2+} fish are significantly lower (P.01) than in the Cd^{2+} treated fish. Statistical analyses were done on all possible relationships between elements of the 0 ppm and 12 ppm Cd^{2+} treated fish and between the 30- and 90-min time intervals. Any probabilities not shown were found to be nonsignificant.

DISCUSSION

From the experimental data presented in this paper, one can conclude that short-term exposure of the cunner to $CdCl_2$ at toxic or near toxic levels does not affect the production of antibody against SRBC. Although this conclusion is based upon early antibody production which, undoubtedly, had not reached a peak, it seems safe to assume that significant differences, which were to appear, would show up as a delay or lag in these early responses. Fish could not be held for long periods of time because of limitations in holding space. Although the second SRBC injection (to hasten the rise in antibody titer) was given after fish had been in Cd^{2+}-free water for 3 days, it is certain that fish still had high Cd^{2+} levels at this point. The data of Greig, Adams, and Nelson (this report, Part II) show continued high levels of Cd^{2+} in cunners after 4 wk of holding in Cd^{2+}-free water. Others have shown that the half-life of Cd^{2+} after a single exposure does is in excess of 200 days in rats, mice, dogs, and monkeys (Friberg, Piscator, and Norberg, 1971, p. 66).

The low-level agglutination titers observed in about half the nonimmunized fish (see titers in Table 1) are not unusual. Natural or nonspecific agglutinins are common among fish, as well as other animals. This does not interfere with immunization experiments as long as these agglutinins are low enough in titer that they are not confused with the results of specific immune stimulation.

In contrast to the results on antibody productio Cd^{2+} did have significant effects on uptake an destruction of bacteria by phagocytes in the liver an spleen. Cadmium at 12 ppm was used in these studi because it was the highest level at which there wa consistent survival of fish during post-Cd^{2+} holdin Fish exposed at this level exhibited a more rapid i itial uptake of bacteria by cells of the liver and splee but a slower bacterial destruction rate than fish n exposed to Cd^{2+}. These results are consistent with co clusions drawn by Holmes, Page, and Good (196 that the metabolic events accompanying phagocytos can be separated into two categories: 1) even associated with particle uptake and 2) even associated with degranulation within the phagocyt In the present study it appears that Cd^{2+} stimulat the metabolic events responsible for bacterial uptak but inhibits degranulation or those events responsib for delivering bactericidal substances to the inte nalized bacteria.

The initial, relatively rapid clearance of bacter from the blood cannot be entirely credited phagocytosis in the liver and spleen since phagocyt cells in the kidney and gill tissues could also co tribute to bacterial uptake and destruction. Howeve cells of the liver and spleen probably take up the m jor portion of the injected antigen. This is assumed f two reasons: 1) in some instances, the liver and splee contained as much as 80% of the total bacteria initia ly injected and 2) studies in other animals indica that cells of the liver and spleen are responsible f removing the majority of intravenously injected pa ticulate antigens. Benacerraf, et al. (1957) found th the liver and spleen of rats removed 85 to 98% of i jected carbon or saccharated iron oxide. McClosk (1972) showed that the liver and spleen of mice retai ed higher proportions of injected bacteria than oth organs. However, data in the latter two referenc show the liver as the organ of major uptake; where in cunners, the spleen usually contains more bacter than the liver. Since the anterior kidney has bee shown to be a site of antibody production in rainbo trout (Chiller et al., 1969), it is assumed that th organ may also take up significant numbers bacteria; however, for the reasons already given, it unlikely that this uptake in the cunner of is the sam magnitude as that of the liver and spleen.

Lack of significant differences between levels bacteria in the liver and spleen at 30 and 90 min (seen in Fig. 1) does not indicate lack of activity with their phagocytic cells. As bacteria are destroye within loaded phagocytes, additional bacteria can b taken from the blood stream to reload the phagocyte Hence, the bacterial levels in these organs may appe to be static when, in fact, there is a rapid turnove The destruction rate of the bacteria (28.7%/hr in th normal fish) can be greatly increased when bacter from 18-hr growth cultures are used (rather than 72-

ultures); in one experiment (unpublished data) able bacteria were reduced so quickly that they opped below the counting range in 30 min. resumably, the older (72-hr) bacteria are in a more ormant state, which is less susceptible to an- bacterial metabolites.

One clue regarding the mechanism of cadmium ac- on is given by this work. It appears that within hagocytic cells cadmium may prevent the delivery of sosomal substances to the phagocytic vacuole or in- bit the action of these substances on bacteria. On he other hand, cadmium does not appear to inhibit ents leading to protein formation. This is indicated cause lymphocytic cells exposed to cadmium in vo actively proliferate and produce antibody protein the same extent as cells not exposed to cadmium.

This work also suggests one way in which cadmium uld reduce fish populations. Since bacteria are more owly killed within phagocytes of cadmium-exposed sh, it follows that certain marginally pathogenic cteria may multiply within phagocytes and even- ally overwhelm the fish with infection. Studies of he effects of chemical agents in other animals suggest common mechanism for phagocytic dysfunction. urenzi et al. (1963) found reduced clearance of rosolized bacteria from lungs of mice exposed to thanol, cortisone, or cigarette smoke. Green and rolin (1967) found that cigarette smoke inhibited e capacity of rabbit pulmonary macrophages to in- tivate bacteria. Kass and Finland (1953) reviewed a rge body of literature showing that treatment of imals with cortisone and other adrenocortical hor- nes increases severity of bacterial, viral, fungal, rotozoan, and helminth infections. Sidransky, erney, and Beede (1965) showed that mice treated th cortisone or cytotoxic cancer therapy drugs came highly susceptible to pneumonia from erosols of *Aspergillus flavus* spores. Merkow et al. 968) then demonstrated that lysosomes in agocytes of cortisone-treated mice failed to fuse th vacuoles containing the spores. Consequently, he substances in these lysosomes were not delivered the vacuole. Jones and Hirsch (1972) have also monstrated absence of lysosomal fusion with agocytic vacuoles containing living toxoplasma arasites. These studies indicate a possible universal chanism for shutting off microbicidal activities hin phagocytes. If so, it is likely that a number of nvironmental pollutants may be found to cause milar phagocytic dysfunction in fish.

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Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium. V. Observations on the Biochemistry

EDITH GOULD and JOHN J. KAROLUS[1]

ABSTRACT

In the liver of cunner, *Tautogolabrus adspersus*, exposed to 3 ppm and to 24 ppm Cd for 96 hr, aspartate aminotransferase activity was 71% and 59%, respectively, of the activity in livers of control fish.

In the livers of cunners exposed to 24 ppm Cd, nicotinamide-adenine dinucleotide reductase activity required 20 m*M* Mg for activation of the same order that 2 m*M* Mg produced in control livers.

Although individual variation precludes generalization here, what may be a metal-complexing group of proteins in the serum of cadmium-exposed cunner warrants further electrophoretic study.

INTRODUCTION

In the collective effort to determine the effects of heavy metals on the marine ecology, relatively little attention has been directed toward possible biochemical malfunctions in marine animal tissue. Yet apart from acute physical trauma, such as gross occlusion of gill tissue, the earliest response of a marine animal to physiological challenge by sublethal concentrations of heavy metals is at the molecular level.

Normal metabolic response to the ingestion or absorption of heavy metals is their temporary inactivation by serum proteins, which sequester and transport the metals to the liver for further processing for removal from the body. Furst, Flessel, and Kelly (1972) observed that noncancer-causing metals, such as zinc and iron, are carried by α- and β-globulins, whereas those heavy metals definitely known to be able to cause cancer, such as nickel and cadmium, are carried by albumins, the major metal-transport protein of blood. Serum electrophoretic patterns may conceivably be a means of detecting an abnormal proportion of metal-protein complexes.

If the normal biochemical mechanisms are unable wholly to inactivate the heavy metals, toxic effects follow. The ionic character of the blood serum becomes seriously deranged (Lewis and Lewis, 1971), with consequent osmoregulatory distress (Thurberg and Dawson, this report, Part III). Key ligand affinities of some enzymes, particularly those for which divalent cations act as positive or negative effectors, can be distorted by a changing ionic environment, with consequent changes in their capacity to react (Gould, 1969, 1971).

Such effects on biochemical systems are most readily assayed by measuring changes in the activity of their constituent enzymes. Because it is not always clear what systems are involved, one must look either to enzymes that are known to sequester metals or to those that require metals for their proper catalytic functioning. Jackim, Hamlin, and Sonis (1970), for example, in their acute-static study of heavy-metal poisoning in the liver of mummichog, *Fundulus heteroclitus,* selected three metal-requiring enzymes (alkaline phosphatase, xanthine oxidase, and catalase), the metal-sensitive ribonuclease, and acid phosphatase, an enzyme involved in mineral metabolism.

In the multidisciplinary study reported here, the cunner, *Tautogolabrus adspersus,* was exposed for 96 hr to varying concentrations of cadmium, a soft Lewis acid with the capacity to bind strongly and irreversibly to sulfur groups. It may be well to note here that the nature of cadmium-protein bonding has been observed to differ with the duration of actual metal challenge (Nordberg, Piscator, and Lind, 1971): in mouse liver shortly after a single injection of cadmium, the cadmium-protein complex was of high molecular weight, whereas in livers of mice surviving for more than 24 hr after injection, the cadmium was bound to a protein of low molecular weight—probably the sulfur protein metallothionein, whose synthesis by the liver is stimulated by prolonged exposure to cadmium (Anonymous, 1972). More importantly, in vitro binding of cadmium by liver homogenates—which would be rapid—is nonselective, and will inhibit sulfhydryl-dependent enzymes, whereas a metallothionein-cadmium complex has no such in-

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hibitory effect. In long-term exposure to sublethal concentrations of heavy metals, therefore, an animal's biochemistry may be adapted in ways that will mask effects observed in acute-static studies.

In the initial seven experiments of this study, results of which are published throughout this collaborative report, skeletal muscle was the only tissue available in sufficient quantity for biochemical testing. Although the relatively slow metabolism of the muscle would not be expected to reflect the biochemical response of rapidly metabolizing liver, for instance, the muscle sarcoplasm was, nevertheless, examined for possible changes in malic enzyme (ME)[2] and α-glycerophosphate dehydrogenase activities, which in vertebrates require manganese and magnesium, respectively, for optimal activity.

Subsequent experiments provided liver and blood, where early reaction to physiological challenge would logically be expected. In one experiment, the test concentrations of cadmium were 0, 3, and 24 ppm, with nine fish at each concentration; the livers were tested for aspartate aminotransferase (AAT) activity, a transaminase that reflects metabolic stress in vertebrates (e.g., Amador and Wacker, 1965) and invertebrates (e.g., Hammen, 1969) alike. In another experiment, only two concentrations of cadmium were used (0 and 24 ppm), with 14 fish at each concentration; the livers of these fish were tested for changes in ligand response of NAD reductase activity. The sera were subjected to electrophoresis and examined for possible changes in total-protein patterns that might reflect an increasing metal-protein fraction, for carbonic anhydrase activity (a zinc-enzyme), and for esterase activity.

METHODS AND MATERIALS

Cunners were exposed to 0, 3, and 24 ppm cadmium for 96 hr in experiments subsequent to those described by Calabrese, Collier, and Miller (this report, Part I), under the same test conditions. The blood was drawn as described by Thurberg and Dawson (this report, Part III); the livers were excised, pooled, and placed in small plastic pouches from which as much air as possible was excluded before freezing. In several of the initial experiments that included more concentrations of cadmium (Calabrese et al., this report, Part I), the fillets were cut from the cunner frames, skinned, and packaged and frozen in the same way as the livers.

Treatment of Tissue

Liver.—The freshly excised livers from three fish were pooled for each sample and were frozen-stored until use, for no longer than 1 wk. The pooled liver were homogenized with a glass pestle in chilled double-distilled water, 1:9 (w/v) for the AAT assay and 1:4 or 1:9 for the NADR-Mg assay. Homogenates were centrifuged for 45 min at 14,500 *g* and 4°C, and the supernates used as the crude enzyme preparation (E). For AAT assays of the freshly frozen livers, it was necessary further to dilute the supernates 1:9 with iced water, for a final E dilution factor of 100.

Skeletal muscle.—Paired fillets, frozen-stored from 4 to 6 wk, were pounded to a rough paste with a iced mortar and pestle and centrifuged for 45 min at 14,500 *g* and 4°C. No suspending medium was used. The centrifuged tissue fluid (CTF) served as the enzyme preparation for ME and αGPdH assays.

Blood.—The clotted fresh blood was clarified by centrifugation for 30 min at 1,720 *g* and 4° C, and the resulting serum was used for electrophoresis.

Assay Procedures

The water used in preparing all solutions was doubly glass-distilled; solutions of substrate and coenzyme were prepared fresh daily; and the assays were read on a double-beam, ratio-recording spectrophotometer, in an optical cuvette with a 1-cm path length. Change in absorbance at 340 nm from 30 to 90 sec after the beginning of the reaction was taken as the unit of measurement ($\Delta A^{340} \times 10^3$/min/0.10 ml E).

Aspartate aminotransferase.—The procedure used was essentially that of Bergmeyer and Bernt (1963), except for the proportions used of reagent solutions. No malic dehydrogenase was added. E, the supernate from the liver homogenate, had a dilution factor of 100.

Protocol:

Buffer-substrate solution: Phosphate buffer (0.1 *M*, pH 7.6), and K aspartate (0.25 *M*)	= 2.70 ml
NADH solution (10 mg/ml H_2O)	= 0.10 ml
E preparation	= 0.10 ml

The solutions were pipetted into an optical cuvette and allowed to stand for 10 min at room temperature. Absorbance was read against a reference cuvette (containing medium with no initial NADH); the reference mixture was adjusted with small increments of NADH so that the difference in absorbance between sample and reference was no greater than 0.600. The reaction was not started until there was no detectable oxidation of NADH. Substrate (0.10 ml 0.2 *M* potassium α-ketoglutarate) was added to start the reaction.

[2] Abbreviations used in this report are: ME=malic enzyme, E.C.1.1.1.40; αGPdH=alpha-glycerophosphate dehydrogenase, E.C.1.1.1.8; NAD=nicotinamide-adenine dinucleotide, and NADH=the reduced form; NADR-Mg=magnesium-dependent NAD reductase activity; and ATT = asparate aminotransferase, E.C.2.6.1.1.

Magnesium-dependent NAD reductase.

Protocol: Tris buffer, 0.1 *M*, pH 9.0	=	1.80 ml
NAD, 13 m*M*, 10 mg/ml H_2O	=	0.10 ml
$MgCl_2 \cdot 6 H_2O$, 0.06 *M* (concn varied)	=	X ml (0, 0.10, 1.00)
H_2O	=	(1.00-X) ml
E to start reaction	=	0.10 ml

The rate of reduction of NAD was followed pectrophotometrically at 340 nm. The control uvette contained everything but the enzyme solu-on.

Malic enzyme.—The assay for ME, a measure of he rate of NADP reduction in the presence of malate, s based on the work of Ochoa et al. (1948), and has een described elsewhere (Gould, 1965). The buffer sed here was Tris, 0.1 *M*, pH 8.0.

α-glycerophosphate dehydrogenase.—This ssay, with a discussion of the cation-dilution chnique, is published in detail elsewhere (Gould, 969).

Electrophoretic Procedures

Electrophoresis.—Electrophoresis of cunner serum ca 3 μl/column) was performed at 4°C using a dis-ontinuous buffer system, on 7% polyacrylamide gel olumns, pH 9.1, with sample and stacker gels of 3% olyacrylamide, pH 5.2. The electrode buffer was Tris 0.005 *M*)-Glycine (0.038 *M*), pH 8.3. Running time as 60 min at 1mA/column followed by 105 min at mA/column, using constant current. Both the gel for-ularies and the electrophoretic procedure are based n the work of Davis (1964) and have been fully escribed elsewhere (Gould and Medler, 1970).

Stains.—For total-protein patterns, the gels were tained with amido schwartz 10B, 1% in 7% acetic cid. They were destained by passive diffusion in several changes of methanol-glacial acetic acid-istilled water (5:1:5), for a total of about 20 hr.

For visualization of esterase sites, the gels were tained with a medium containing α-naphthyl utyrate (50 mg in 2 ml acetone to dissolve, then 2 ml H_2O), coupled with Fast Garnet GBC in 46 ml hosphate buffer, 0.1 *M*, pH 7.0. Polyvinylpyrrolidone PVP) (ca 500 mg) was added to the buffer to aid solubilization of the dye. The substrate was added to he dye-PVP-buffer solution immediately prior to use, nd the whole filtered through glass wool. Incubation was in the dark at room temperature for 45 min.

RESULTS AND DISCUSSION

In homogenates of fresh-frozen livers from cunners exposed to cadmium, AAT activity was significantly lower than in the controls (Table 1). Livers of fish exposed to 3 ppm Cd had only 71% of the AAT activity observed in livers of control fish and in fish exposed to 24 ppm Cd, activity dropped to 59% of the control. Whether this cadmium-induced drop in activity represents a simple enzyme block, a depression of microsomal biosynthesis, or a more involved mechanism of inhibition cannot be speculated from these few data; the observations here serve only to indicate possibly profitable areas for further work. Parenthetically, activity of this transaminase in fresh-frozen livers had roughly 10 times the activity of livers frozen-stored for longer than 1 wk. Livers frozen for 2-8 wk (at ca +5°C) not only had much lower AAT activity than the fresh livers, but also had widely variable rates of loss of activity.

Another fresh cunner-cadmium experimental series, with 14 fish at 0 ppm Cd and 14 at 24 ppm Cd, provided livers for a study of what appears to be a soluble, magnesium-linked NAD reductase (NADR-Mg). Initially, the assay was intended to be for α-GPdH, but it was discovered that the magnesium effect was stronger without the αGP substrate: in the presence of αGP(10 m*M*), 2 m*M* Mg produced only a 1.2% and 20 m*M* only a 1.8% increase in reductase activity over activity with no added magnesium; whereas with no added substrate, 2 m*M* Mg produced 58% and 20 m*M* produced 130% increase in reductase activity over that with no added magnesium (Table 2). The endogenous substrate pool might be expected to contribute a strong variable to NADR activity (although not so much in teleosts as in marine in-

Table 1.—Aspartate aminotransferase activity in liver of cunner, *Tautogolabrus adspersus*, exposed for 96 hr to varying concentrations of cadmium, 25 ppt salinity. Each value is the change in absorbance at 340 nm for 1 min under assay conditions and represents the average of 2 tests. Each sample is a pool of livers from three fish; the enzyme preparation (E) has a 100× dilution factor.

Test concn Cd (ppm)	AAT activity ($\Delta A^{340} \times 10^3$/min/ 0.10 ml E)
0 ppm Cd	151.5
	170.0
	152.5
3 ppm Cd	110.0
	115.0
24 ppm Cd	84.0
	93.5
	101.5

Table 2.—Effect of added Mg^{2+} on NAD reductase activity with and without added α-glycerophosphate. E dilution factor is 10×. Sample is pool of three control livers of cunners, *Tautogolabrus adspersus*.

Final concn Mg^{2+} (m*M*)	NAD reductase activity ($\Delta A^{340} \times 10^3$/min/0.10 ml E) without added substrate	with α-GP (10 m*M* final conc)
0	5	41
2	29	48
20	65	75

vertebrates), but the assay protocol here was designed to reduce that variable as much as possible, by using the enzyme activity without added magnesium as a base from which to measure magnesium activation. Table 3 lists data showing a mild cadmium effect upon the magnesium activation of cunner liver NADR: in fish exposed for 96 hr to 24 ppm Cd, an approximately 10-fold increase in magnesium concentration (20 m*M*) is required to activate NADR as much as 2 m*M* Mg does in the control fish.

In frozen-stored cunner skeletal muscle, what appeared to be a significant difference in ME properties of pooled fish exposed to cadmium, as contrasted with pooled controls, must be ascribed to individual variation. αGPdH activity was very high, but there was no apparent difference in properties between the cadmium-exposed and the control fish.

Electrophoretic studies are as yet inconclusive. Despite considerable variation in the total-protein pattern, there is an area of tentatively labeled metal protein complexes that appeared in most cases (series out of 10) to increase in fish that had been ex posed to cadmium (Fig. 1); many more data would b necessary to establish statistical validity, howeve and individual variation renders this approac questionable in an acute-static study. Detoxificatio mechanisms of this nature may be more prominent i a chronic study.

Of the serum enzymes tested for isoenzyme varia tion, only α-naphthyl butyrate esterase activit produced a difference in pattern between cadmium exposed and control fish; but here again, individua variation was very strong.

On the whole, the observations made in the cours of this preliminary experimentation seem to point t further work with metal-activated enzymes and wit the stress-indicator transaminases. The most clear cut effects were obtained by using assays designed t measure the degree of ligand activation or inhibition

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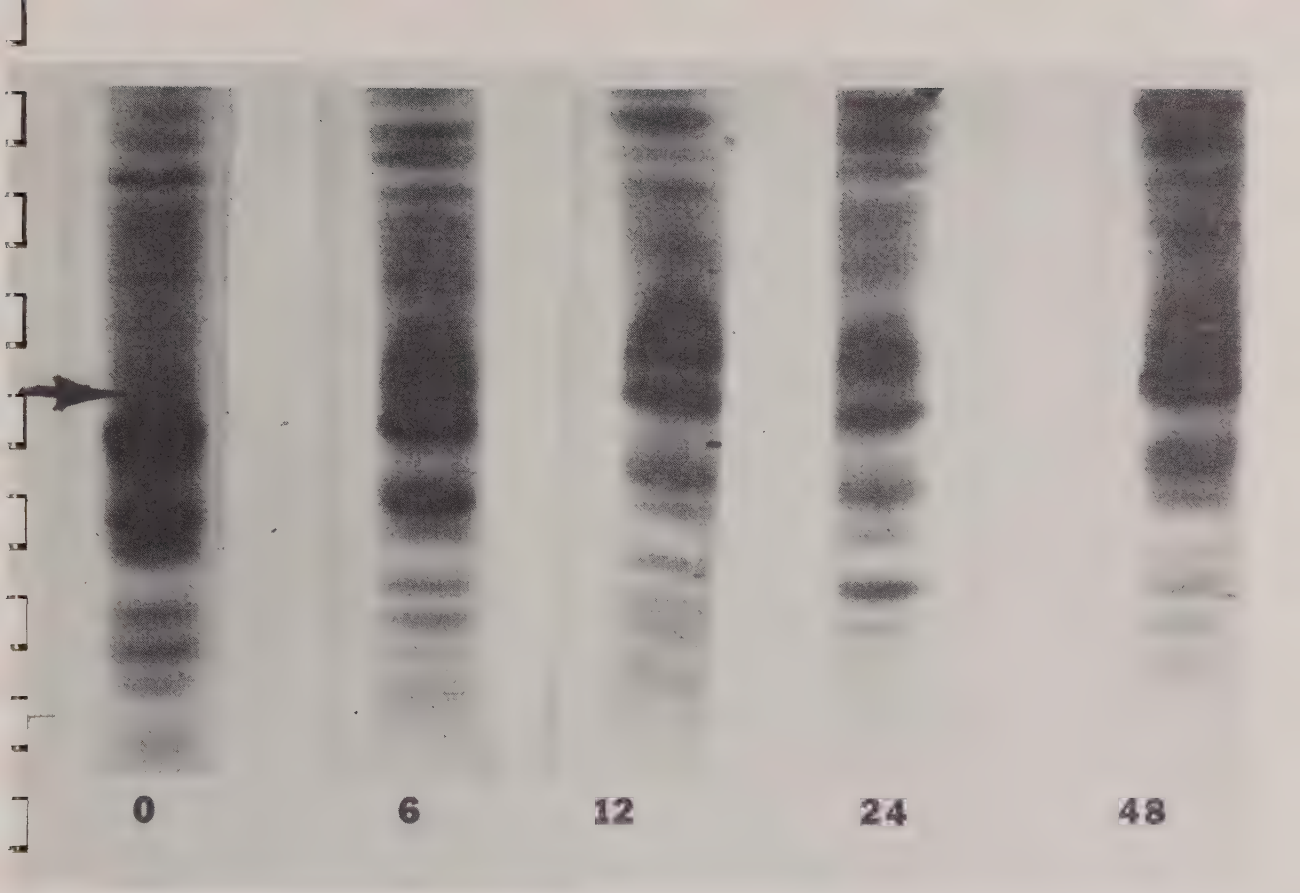
Table 3.—Mg-dependent NAD reductase activity in liver of cunner, *Tautogolabrus adspersus*, exposed for 96 hr to 24 ppm Cd, 25 ppt salinity. Each value is the average of two tests, and each sample is a pool of three livers.

Homogenate dilution factor	Increase in activity ($\Delta A^{340} \times 10^3$/min) effected by addition of Mg^{2+} to assay medium: 0 ppm Cd, final concn Mg^{2+} (m*M*) 0 (Init. Δ A)		2	20	24 ppm Cd, final concn Mg^{2+} (m*M*) 0 (Init. Δ A)		2	20
10×	0	(5)	24	60	0	(13)	1	35
10×	0	(9)	29	54	0	(22)	0	26
5×	0	(21)	10	83	0	(23)	4	32
5×	0	(22)	35	56	0	(18)	12	27
5×	0	(23)	26	57	-		-	-
5×	0	(18)	20	88	-		-	-
5×	0	(20)	24	51	-		-	-
5×	-	-	-	-	0	(2)	10	45



gure 1.—Protein pherogram of serum from cunners, *Tautogolabrus adspersus*, exposed for 96 hr to 0, 6, 12, 24, and 48 ppm cadmium chloride, at 25 ppt salinity. Arrow points to area tentatively considered to comprise metal-protein complexes.

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Physiological Response of the Cunner, *Tautogolabrus adspersus*, to Cadmium. VI. Histopathology

MARTIN W. NEWMAN and SHARON A. MacLEAN[1]

ABSTRACT

The histopathological effects of acute exposure of cunner, *Tautogolabrus adspersus*, to water containing cadmium chloride were manifested in the kidney, intestine, hemopoietic tissue, epidermis, and gill. Few significant changes were noted in fish exposed to concentrations less than 48 ppm for 96 hr. The results implicate renal failure as the probable cause of death after acute exposure to cadmium.

INTRODUCTION

The histopathological aspects of this study were undertaken in the hope of contributing to our nowledge of the effects of heavy metals at the level of e cells and tissues. Elucidation of the mechanisms of observed physiological and behavioral responses, nd development of baseline information useful for in- rpretation of specimens which may be collected om naturally occurring mortalities were further goals. Only Gardner and Yevich (1970) have stematically examined blood and tissues of a teleost posed to cadmium. While the exposure levels used that study and the present one were similar, the ish species and the length of exposure (96 vs. 48 hr) ere different.

MATERIALS AND METHODS

Techniques used in handling the cunner, *autogolabrus adspersus,* in this study have been described by Calabrese, Collier, and Miller (this eport, Part I). Blood smears were prepared from art blood (see Thurberg and Dawson, this report, art III) and stained by Giemsa or Wright's methods. Differential white cell counts were performed using he first 250 leucocytes encountered on each smear. ll thrombocytes seen while counting leucocytes were so enumerated. Tissue samples were removed for physiological and biochemical studies (Thurberg and awson; Gould and Karolus, this report, Parts III and respectively). The remainder of each fish was fixed Davidson's (AFA) fixative for 48-96 hr then ransferred to 70% EtOH. Tissues were embedded in raffin, sectioned at 6 μm, and stained with a variety techniques including Giemsa, PAS, PAS-Alcian Blue, Mallory triple-stain, and Perl's Prussian blue reaction. Tissues from each of six fish exposed to 0, 6, 12, 24, and 48 ppm cadmium, or a total of 30 fish were examined.

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RESULTS

Intestine

Pathological changes were seen in the intestinal epithelium of cunners exposed to high concentrations of cadmium. In the 24 ppm exposure group, there was some swelling of the intestinal epithelium. At 48 ppm, five of six fish exhibited varying degrees of pathological change. The columnar cells were swollen. Nuclei were hypertrophied and occupied a position farther from the basement membrane than those of unexposed fish. Nucleoli became very prominent. Numbers of mucus secreting cells appeared about equal to or slightly less than in the control animals. In two of the above five fish, the intestinal epithelium was sloughed from the basement membrane in many places and the lumen contained much cellular debris and mucus (Fig. 1).

Kidney

One of six fish exposed at the 24 ppm level had some cloudy swelling in a few scattered areas of the proximal tubules. At 48 ppm, the kidneys of five of six fish examined showed some degree of pathological change. Three of the fish exhibited diffuse tubular necrosis (Fig. 2), one exhibited focal tubular necrosis, and one only a few scattered necrotic lesions. The proximal segments of the tubules appeared to be most affected. The lumina of more distal areas of the tubules often contained sloughed epithelial cells or were filled with a hyaline eosinophilic material (Fig. 3).

Glomeruli appeared normal. The blood spaces in kidneys of fish exposed to 24 and 48 ppm cadmium

Figure 1.—Intestinal epithelium of a control fish (a) and a fish exposed to 48 ppm cadmium for 96 hr (b). Necrosis and sloughing o the intestinal epithelium is evident in the experimental animal. Note also that much cellular debris occupies the lumen. 120× Mallory's triple stain.

contained large numbers of cells thought to be immature thrombocytes (Fig. 4). The occurrence of erythrophagocytosis and of hemosiderin, common in the kidneys of control fish, was reduced or absent in the kidneys of the 48 ppm group (Fig. 5).

Gills

The appearance of the gills of cadmium-exposed fish was quite variable. The following defects were noted in decreasing order of prevalence: epithelial hypertrophy, hyperplasia of interlamellar epithelium, and desquamation. These changes were noted at all levels of exposure. The gill tissue of some heavily exposed fish appeared normal and some lesions were seen in the control animals. Some of these lesions may represent postmortem changes. In view of the extensive variability of gill lesions which could not be correlated with exposure levels, little emphasis was placed on the appearance of this tissue.

Epidermis

The epidermis appeared normal in fish exposed at levels up to 24 ppm. At 48 ppm swelling of the epithelial cells and a paucity of mucus secretion was noted (Fig. 6).

Blood

Obvious qualitative differences noted between smears from cunner exposed to 24 and 48 ppm cadmium and control fish consisted of poikilocytosis and karoklasis (Figs. 7 and 8).

Differential leucocyte counts revealed throm bocytopenia and lymphocytopenia. However, th percentage of neutrophils increased (Table 1).

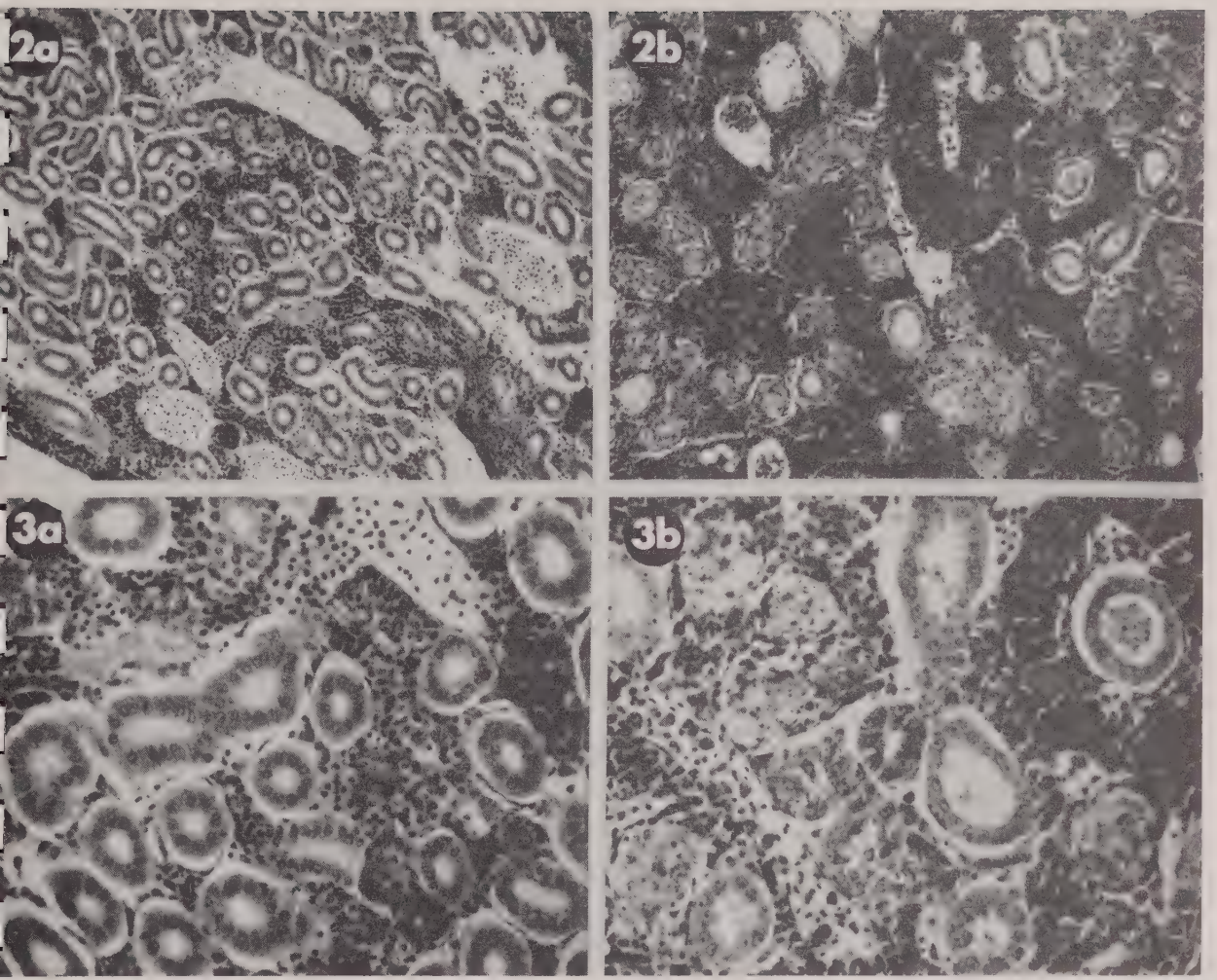
DISCUSSION

The histological and hematological response of a estuarine teleost to cadmium has been studied b Gardner and Yevich (1970) using mummichog, *Fun dulus heteroclitus*. Their experiments involved acut exposure to 50 ppm cadmium for up to 48 hr. Th findings of the present study are very like those c Gardner and Yevich. Discrepancies which did aris might be related to difference in the fish species used or length of exposure.

Changes in the intestine of cunners were similar t those reported in mummichogs. Gardner and Yevic (1970) found an increase in mucus cell activity whil the present study indicates a normal or slightl depressed level of activity. This difference may be at tributable to the increased exposure time of cunner Lymphocytic infiltration of the submucosa was no observed in the present study.

Kidney

Morphologic changes in the kidney of teleost exposed to cadmium have also been documented b Gardner and Yevich (1970). Pathological changes i the kidneys of mammals exposed to cadmium are we known. Foster and Cameron (1963) produced rena lesions in rabbits with two subcutaneous injections c $CdCl_2$ (9 mg Cd^{2+}/kg). These lesions were limited t



igures 2 and 3.—Kidney of control fish (a) and of fish exposed to 48 ppm cadmium for 96 hr (b). Note diffuse tubular necrosis in 2b. ubular epithelium in various stages of degeneration can be seen in 3b. Note the swollen nuclei of still intact epithelial cells and ellular debris in the dilated lumina. 120× (2), 300× (3); Azure-eosin stain.

Table 1.—Effects of 96-hr exposure to cadmium chloride on differential leucocyte counts of cunners, *Tautogolabrus adspersus* (figures are averages, standard deviation in parenthesis).

Cadmium exposure (ppm)	No. sampled	Leucocytes (%)						
		Mature thrombocytes	Neutrophils	Small lymphocytes	Medium lymphocytes	Eosinophils	Blasts	Monocytes
0	8	69.3 (±10.8)	15.6 (±9.1)	11.2 (±3.9)	2.2 (±0.7)	0.7 (±0.7)	1.1 (±1.2)	0
3	9	65.3 (±10.1)	18.5 (±10.3)	10.3 (±4.9)	2.4 (±1.4)	0.6 (±0.4)	2.9 (±3.3)	0
6	8	60.5 (±16.3)	23.7 (±19.1)	9.3 (±5.6)	2.3 (±2.3)	1.2 (±0.5)	2.3 (±1.7)	< 0.1
12	9	65.2 (±16.5)	24.1 (±16.3)	7.5 (±4.9)	1.3 (±1.0)	0.6 (±0.5)	1.6 (±0.5)	< 0.1
24	9	56.3 (±20.8)	32.1 (±19.3)	6.4 (±4.6)	1.6 (±0.9)	0.6 (±0.6)	3.0 (±2.6)	< 0.1
48	10	35.9 (±12.4)	50.2 (±12.4)	4.5 (±4.8)	1.5 (±1.1)	1.4 (±1.1)	6.1 (±4.8)	0

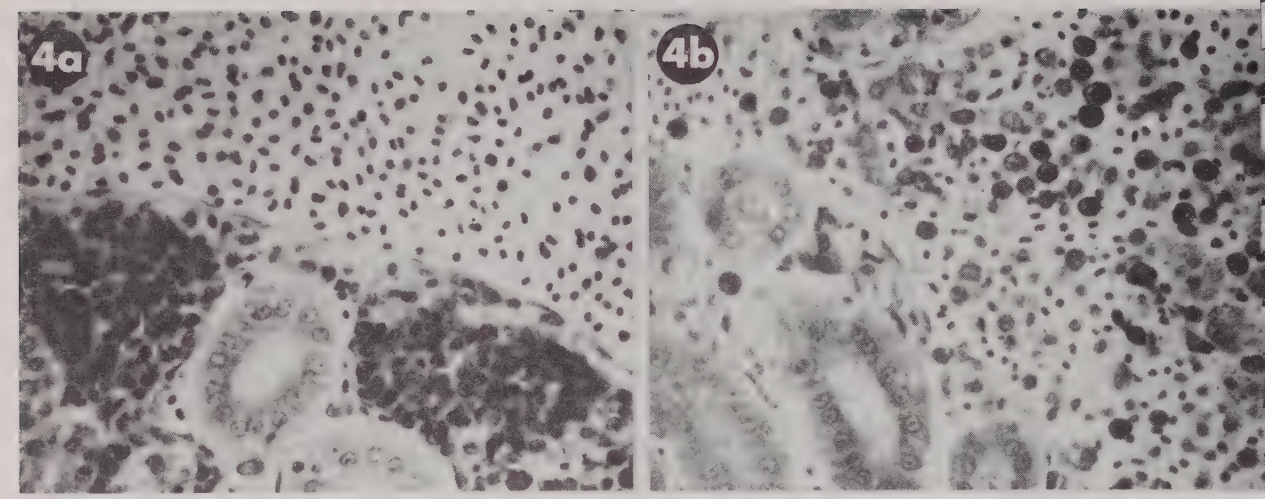


Figure 4.—Kidney of control fish (a) and of fish exposed to 24 ppm cadmium for 96 hr (b). Note difference in the cell population occupying the vascular spaces. 480×; Azure-eosin stain.

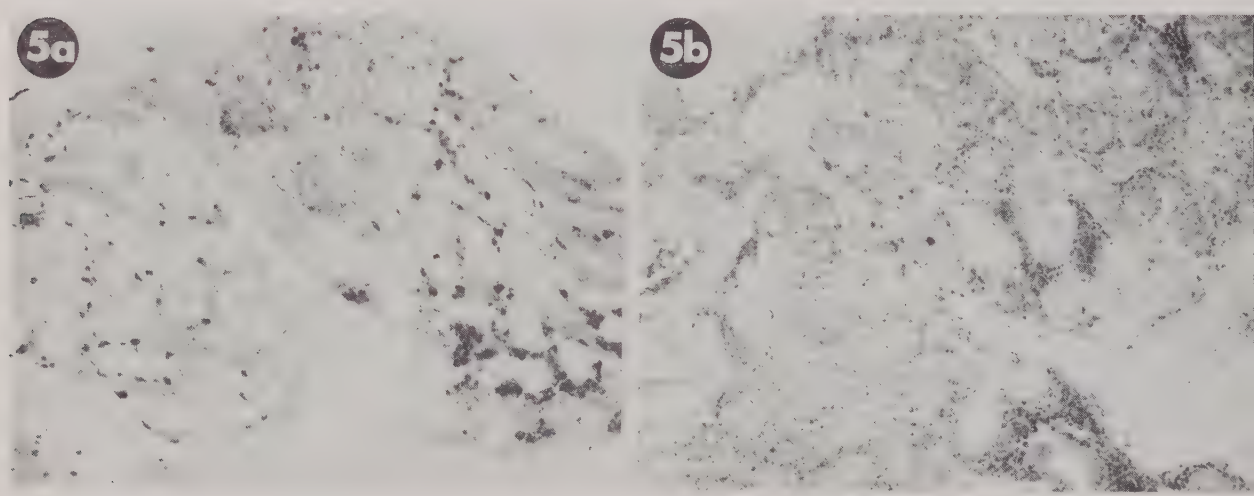
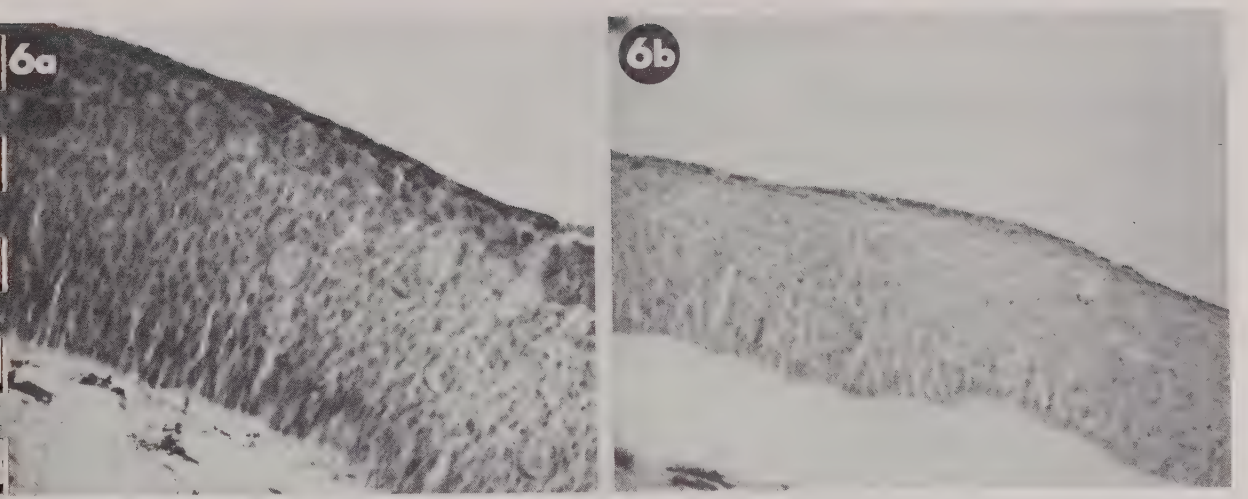


Figure 5.—Kidney of control fish (a) and of fish exposed to 48 ppm cadmium for 96 hr. An abundance of hemosiderin can be see in the control fish. 120×; Perl's stain.

the proximal tubules. Similar experimental results were obtained by Dalhamn and Friberg (1957); Ahlmark et al. (1961) found decreased glomerular filtration rates in human workers exposed to cadmium. Proteinuria is another common finding in human cadmium workers (Friberg, 1950; Piscator, 1962). Proteinuria occurs subsequent to tubular dysfunction because of decreased protein reabsorption in the proximal tubule.

During chronic exposure or early in acute exposure, cadmium is concentrated in the renal cortex in the form of metallothionein, a cadmium- or zinc-containing protein with a molecular weight of about 10,000 (Kägi and Vallee, 1960). When cadmium concentrations in renal cortex reach approximately 20 ppm wet-weight, functional and morphologica change occurs in the kidneys of rabbits, rats, and me (Friberg, Piscator, and Nordberg, 1971). After ad ditional exposure, the functional changes result in th failure of the kidney to reabsorb the metallothionein and increased excretion of cadmium and a lowering the concentration of the metal in the kidney occurs

The effect of cadmium poisoning on the amount hemosiderin in the teleost kidney has not bee previously described. Unlike most homeotherm many teleosts normally have a great deal of pigmen in the kidney, composed of both lipofuscin an hemosiderin. The observation of reduced pigment i



ure 6.—Epidermis of control fish (a) and of fish exposed to 48 ppm (b). Note nuclear hypertrophy and paucity of mucus secreting ls in 6b. The separation of the epidermis from the deeper layers is probably sectioning artifact. 120×; PAS-hematoxylin stain.

ne cunners exposed to 48 ppm cadmium may be used by the metal's effect on the hemopoietic stem. Friberg (1950) found no effects in human bone narrow after respiratory exposure. Wilson, DeEds, nd Cox (1941) found that rats fed a diet containing as v as 31 ppm cadmium would be anemic. By administering iron to rats receiving cadmium in their iet, anemias were reduced (Friberg, 1955). This indicated that cadmium was not directly blocking moglobin synthesis, but might be interfering with e uptake of iron by the intestine. If a reduction of on absorption was occurring in the cunner, this, ong with the observed decrease in erythrophagocytic tivity, might account for the observed reduction in emosiderin in exposed animals.

ill

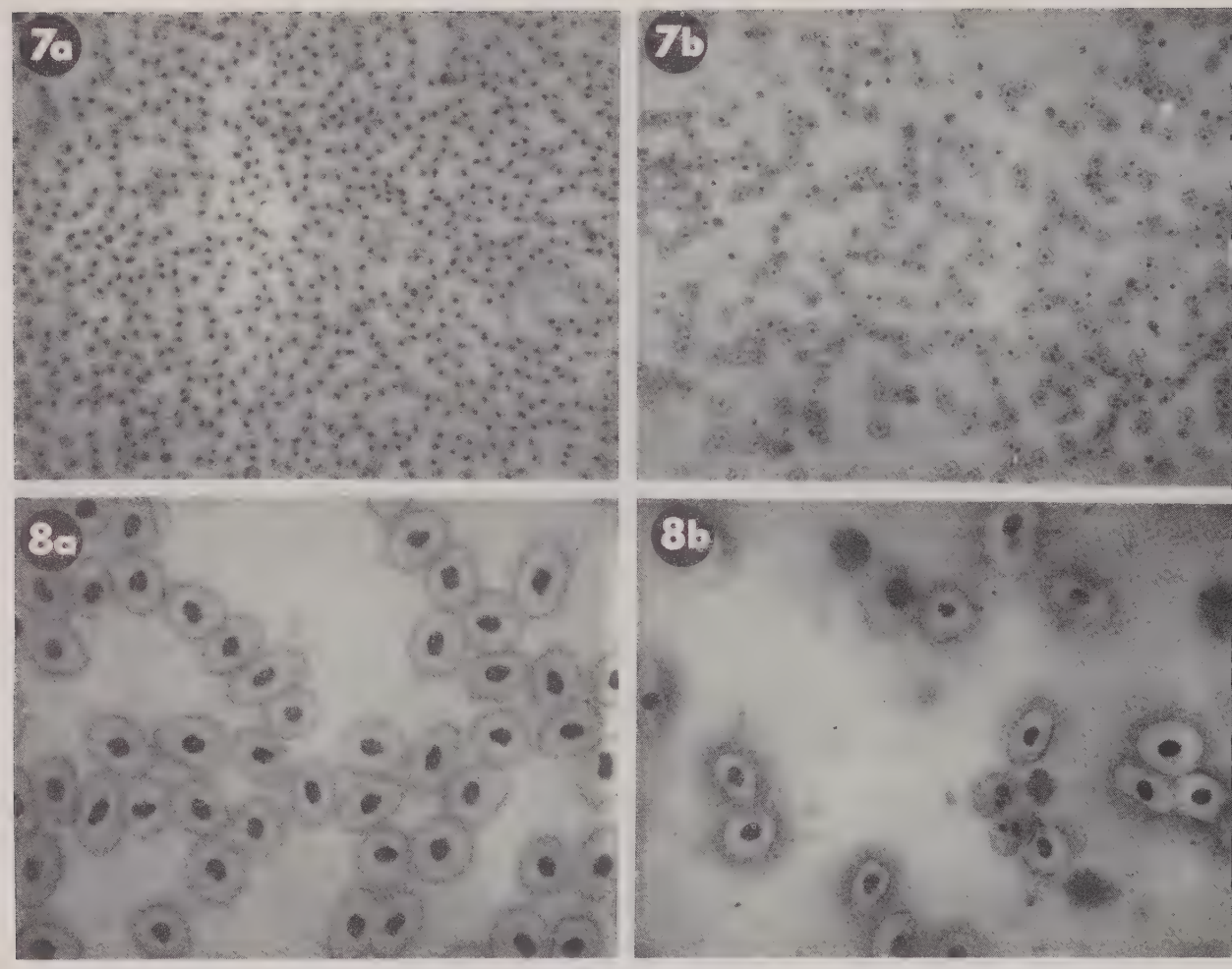
The lack of correlation between exposure level and bserved pathologic changes in the gill tissue can be plained by Gardner and Yevich's observations on ummichogs. They noted that gill lesions were focal n that all gill filaments were not involved, and the esions were of a random nature, not limited to ecific areas of the branchial arches.

The importance of the effects of heavy metals on he respiratory epithelium of fishes is not clear. Skidore (1964) believed that death of rainbow trout, *lmo gairdneri,* exposed to acutely toxic solution of nc sulphate was caused by tissue hypoxia, resulting rom the damaging effect of the metal on gill ithelium. Subsequent experimental studies seem to nfirm this hypothesis (Skidmore, 1970). Burton, Morgan, and Cairns (1972) studied levels of lactic and yruvic acid in muscle and liver of rainbow trout exsed to acute zinc toxicity and reached conclusions nilar to Skidmore. Schweiger, cited in Eisler (1971), oncluded that the toxic action of cadmium on several freshwater fish and invertebrates was due to suffocation. Friberg et al. (1971) cite examples of respiratory exposure to cadmium producing lung damage in mammals at levels which were insufficient to cause kidney damage. Doudoroff and Katz (1953) are cited by Mount and Stephan (1967) as listing nine references attributing the death of fish in solutions containing heavy metal salts to coagulation of mucus on the gill or damage to gill tissue. Bilinski and Jonas (1973) found that exposure of rainbow trout to 11.2 ppm Cd^{2+} for 72 hr resulted in a 66% mortality, but there was no significant change in the ability of the gill tissue to oxidise lactate.

Evidence from the present study implicates renal failure as a cause of death in cunner after acute exposure to cadmium. Although pathological changes were observed in gill tissue and gills exposed to even low levels of cadmium seemed to sustain some physiological damage (Thurberg and Dawson, this report, Part III), these changes do not seem to be asociated with mortality. On the other hand, those fish exposed to 48 ppm cadmium for 96 hr all died within a few days after being returned to clean seawater (Calabrese, Collier, and Miller this report, Part I). Therefore, as regards the effects of cadmium on the cunner, mortality seems to be associated with severe pathological changes of an apparently irreversible nature taking place in the kidney.

Epidermis

The destruction of the mucus cells of the epidermis could have important consequences as it would eliminate the fish's first line of defense against infectious microorganisms. The possibility that heavy metals would destroy mucus cells with exposures too short to cause renal damage should be investigated. In the present study, the fish had already received a



Figures 7 and 8.—Blood smear of control fish (a) and fish exposed to 48 ppm cadmium for 96 hr (b). Note poikilocytosis, karyolka and abundant smudge cells in 7b, 8b. 185× (7), 750× (8); Wright's stain.

lethal exposure to the metal so that destruction of the mucus cells was not an important factor in prognosis.

Blood

An understanding of the changes taking place in the blood of the cunner must await further study. The increase in eosinophils noted by Gardner and Yevich (1970) was not seen, possibly because of the longer exposures used in this study. Wilson et al. (1941) noted an increase of eosinophils of rats after dietary exposure to cadmium, and Friberg (1950) found an increase of eosinophils in rabbit blood from a normal 3% level to 25% after exposure to cadmium oxide dust.

Thrombocytopenia, lymphocytopenia, and neutrophilia in the present study parallel the changes found by Gardner and Yevich (1970) in mummichogs and have also been found in other species under stress or after adrenal corticoid administration (Weinreb, 1958).

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